The use of methylene blue as an extravascular surgical marker impairs vascular responses of human saphenous veins

Methylene blue is occasionally applied to the adventitia of blood vessels during coronary artery bypass and other vascular procedures to assist in the orientation of the vessel. Inherent in this method is the assumption that extravascular application of methylene blue is innocuous with regard to vascular function. In the first part of this study, the *in vitro* vascular reactivity of methylene blue-labeled saphenous veins was compared with that of veins that were not marked with methylene blue. The vasoactive agents tested were designed to examine multiple pathways. They included potassium chloride, prostaglandin F_{2α}, phenylephrine, serotonin, angiotensin II, BHT-933 (α₂-adrenergic agonist), sodium nitroprusside, acetylcholine, isoproterenol, and verapamil. Compared with unmarked veins, those marked with methylene blue demonstrated a significant impairment of both vasoconstrictor and vasodilator function. These observations were made on a relatively small number of patients and could therefore be attributed to inherent differences between patients or surgical procedures. In the second part of this study, these variables were eliminated by dividing a single vein from one patient into three segments for a 45-minute exposure to external only methylene blue, internal and external methylene blue, or no methylene blue. The segments were then evaluated for vasoreactivity *in vitro*. Externally applied methylene blue reduced vasoconstriction regardless of the agonist. Further, both endothelium-dependent and -independent vasodilation was diminished by external methylene blue exposure. In veins exposed to methylene blue both internally and externally the results were similar but the magnitude of impairment greater. It is concluded that surgical marking of blood vessels with methylene blue has the potential to adversely affect vascular reactivity and therefore the use of alternative dyes should be considered. (J THORAC CARDIOVASC SURG 1995;109:21-9)

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Methylene blue (MB) is occasionally applied extravascularly during a variety of vascular proce-

dures to assist in the orientation of the blood vessel. The use of marking dyes serves to facilitate isolation and may therefore reduce surgical trauma. This is particularly true with microvascular operations in which vascular torsion can be reduced by the use of marking dyes.¹ Procedural trauma to blood vessels is a legitimate practical concern, especially when considering the functional importance of the delicate endothelium. The use of MB as a marking dye is thought to be innocuous on the basis of the assumption that application to the adventitia is unlikely to significantly penetrate the smooth muscle or endothelium. However, a recent report by Mazmanian and associates² suggests that MB applied to the adventitia has significant pharmacologic actions in rat tail arteries at doses 100 times lower than the

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Fig. 1. Maximal vasoconstrictor responses measured in grams of tension in saphenous veins which were unmarked (*open column*, no MB, $n = 12/72$) or marked (*black column*, MB, $n = 5/24$) with MB during operations in which coronary artery bypass is used. **A**, Constriction elicited by membrane depolarization induced by KCl, 100 mmol/L. **B**, Constriction elicited by exposure to PGF_{2α}, 3 μmol/L. No significant differences were detected.

conventional 1% MB stock solution used for surgical marking. Further, Bentz and coworkers³ used an in vitro human arterial model to demonstrate that topical MB may increase platelet deposition. Therefore, careful reevaluation of MB as a surgical marker seems prudent. In this study, we evaluate the potential of MB to affect the in vitro vascular reactivity of human saphenous veins using two independent approaches.

Materials and methods

Vascular procurement. Sections of saphenous vein were randomly obtained from 23 patients undergoing coronary artery bypass grafting. The vessels were immediately transported to the laboratory in a modified Krebs-Henseleit buffer of the following composition: NaCl, 118.0 mmol/L; KCl, 4.7 mmol/L; MgSO₄, 1.2 mmol/L; KH₂PO₄, 1.1 mmol/L; CaCl₂, 2.5 mmol/L; NaHCO₃, 25.0 mmol/L; dextrose, 10.0 mmol/L; indomethacin, 2.8 μmol/L; and sodium ethylenediaminetetraacetic acid, 10 μmol/L; 25° C, pH 7.4, previously aerated with 95% oxygen and 5% carbon dioxide, and sealed. The vein was carefully cleaned of all surrounding fat and connective tissue and cut into 4 mm rings. The vascular rings were suspended between two metal hooks in a 10 ml tissue bath containing identical buffer at 37° C continuously aerated with 95% oxygen and 5% carbon dioxide. The vascular isometric tension was measured with a Grass FT 03 force-displacement transducer (Grass Instrument Co., Quincy, Mass.) integrated to a low-level direct-current amplifier recording on a paper-charted polygraph (Beckman R611; Beckman Instruments, Inc., Fullerton, Calif.). The rings were stretched to optimal diastolic resting tension as determined in pilot studies (2 gm) and allowed 45 minutes to equilibrate before initiation of protocols. Bath solutions were changed every 20 minutes throughout all experiments. The time between tissue harvest and initiation of protocols was less than 3 hours.

Protocols. For the first set of experiments, responses of saphenous veins that had been marked with MB ($n = 5/24$) in the operating room were compared with unmarked veins ($n = 12/72$). After equilibration, all rings were constricted with KCl at a concentration of 100 mmol/L and then washed. Vasoconstrictor dose response curves were obtained by exposing each ring to increasing concentrations of only one of the following: phenylephrine, serotonin, angiotensin II, or the α₂-adrenergic agonist BHT-933 (BHT). After washout, the vessels were precontracted with prostaglandin F_{2α} (PGF_{2α}) 3 μmol/L. Vasodilator dose response curves were then obtained by exposing each vascular ring to increasing concentrations of only one of the following: sodium nitroprusside, acetylcholine, isoproterenol, or verapamil. According to this design, each ring was exposed to only one vasoconstrictor and one vasodilator (plus KCl and PGF_{2α}). The order of drug administration was randomized.

For the second set of experiments, an unmarked saphenous vein was cut into three segments, each 3 to 4 cm long ($n = 6/36$). One segment was tightly ligated with suture at both ends. The ligated segment and an unligated segment were placed in continuously aerated Krebs buffer containing 0.1% MB (2.7 mmol/L). This dose of MB is 10 times lower than the conventional MB solution used to mark veins in the operating room. The remaining venous segment was placed in an identical buffer without MB. After 45 minutes, the segments were removed and extensively washed with Krebs solution. The exposure time was based on the estimated average time that a vein is exposed to MB from the time of marking to transplantation during a bypass procedure. Two vascular rings from each segment were cut and suspended in the tissue bath as described earlier. The ligated vessels that had been placed in the MB buffer served as the "external MB" group. The unligated vessels exposed to MB buffer and the unligated vessels exposed to MB-free buffer served as the "internal/external MB" group and the "MB-free" group (control), respectively. After equilibration, all rings were constricted with KCl 100 mmol/L and then washed. Vasoconstrictor dose response curves were obtained by exposing each ring to

Fig. 2. Vasoconstrictor dose response curves for phenylephrine (A), serotonin (B), angiotensin II (C), and BHT (D) obtained from saphenous vein ring preparations that were unmarked (open circles, no MB, $n = 12/18$) or marked (black circles, MB, $n = 5/6$) with MB during operations for use in coronary artery bypass. Symbols on the X-axis depict EC_{50} values for the respective curve. $*p < 0.05$.

increasing concentrations of either phenylephrine or serotonin. After washout, the vessels were precontracted with $PGF_{2\alpha}$ 3 $\mu\text{mol/L}$ and vasodilator dose response curves were obtained by exposing each ring to increasing concentrations of either sodium nitroprusside or acetylcholine. As with the previous design, each ring was exposed to only one vasodilator. Gross and histologic examination at the conclusion of the experiment showed no visible MB on the endothelial surface of the ligated vessels.

Data analysis. When enough tissue was available, the rings were run in pairs and the responses averaged to yield a single value. Therefore, all n values are presented as the number of patients followed by the number of ring preparations. In all cases, statistical analysis was performed with the number of patients used as the n value. MB impaired the ability of veins to constrict to both KCl and $PGF_{2\alpha}$. This impairment can potentially skew any data normalized to KCl or $PGF_{2\alpha}$ responses. Therefore, vasoconstrictor responses are reported both in grams of tension and as a percentage of response to KCl. Likewise, vasodilator responses are presented as both a percentage of precontraction and as grams of relaxation. Dose response curves were evaluated by an analysis of variance with a Scheffe's adjustment for multiple comparisons. For the first set of experiments in which the veins were from different patients, an unpaired t test was used to assess single KCl and $PGF_{2\alpha}$ responses. In the second set of experiments in which veins for each test group were from

the same patient, a paired t test was used to compare the single KCl and $PGF_{2\alpha}$ responses to control responses. Effective concentrations for half-maximal responses (EC_{50}) were calculated by performing a curve fit on each individual vascular ring's dose response. The equation chosen for curve fitting was based on receptor theory and is detailed elsewhere.⁴ Log-normal distribution is expected for equieffective doses; therefore, mean logs were used in comparing EC_{50} values as previously described.⁵ The EC_{50} values were compared by an unpaired t test in Table I and an analysis of variance with Scheffe's test in Table II. All data are presented as means plus or minus standard error of the mean and only those differences with a p value of less than 0.05 were considered statistically significant.

Results

Veins marked with MB during the operation. A total of 12 of 72 unmarked (from 8 men and 4 women) and 5 of 24 MB-marked (from 3 men and 2 women) saphenous vein preparations were evaluated for vascular reactivity. Compared with unmarked vessels, those marked with MB showed a significant impairment in their ability to contract to all vasoconstrictor agents used (Figs. 1 and 2). Vasoconstriction to KCl (Fig. 1, A) and $PGF_{2\alpha}$ (Fig.

Fig. 3. Vasodilator dose response curves for sodium nitroprusside (A), acetylcholine (B), isoproterenol (C), and verapamil (D) obtained from saphenous vein ring preparations that were unmarked (open circles, no MB, $n = 12/18$) or marked (black circles, MB, $n = 5/6$) with MB during operations for use in coronary artery bypass. Responses are expressed as a percentage of precontraction obtained with PGF_{2α} 3 μ mol/L. Symbols on the X-axis depict EC₅₀ values for the respective curve. * $p < 0.05$.

Table I. EC₅₀ values for saphenous veins marked with methylene blue during surgery compared with veins not marked

Drug	EC ₅₀ MB free ($n = 5$)	EC ₅₀ MB marked ($n = 12$)
Phenylephrine	$(9.6 \pm 5.2) \times 10^{-7}$ mol/L	$(7.1 \pm 1.8) \times 10^{-7}$ mol/L
Serotonin	$(8.2 \pm 4.5) \times 10^{-8}$ mol/L	$(1.6 \pm 0.9) \times 10^{-7}$ mol/L
Angiotensin II	$(5.1 \pm 9.1) \times 10^{-9}$ mol/L	$(9.9 \pm 10.3) \times 10^{-9}$ mol/L
BHT-933	$(1.4 \pm 1.7) \times 10^{-6}$ mol/L	$(8.7 \pm 3.5) \times 10^{-7}$ mol/L
Sodium nitroprusside	$(1.8 \pm 0.7) \times 10^{-7}$ mol/L	$(1.7 \pm 0.9) \times 10^{-6}$ mol/L*
Acetylcholine	$(4.7 \pm 2.3) \times 10^{-8}$ mol/L	$(4.5 \pm 4.7) \times 10^{-7}$ mol/L*
Isoproterenol	$(3.1 \pm 1.9) \times 10^{-8}$ mol/L	$(2.0 \pm 0.9) \times 10^{-7}$ mol/L*
Verapamil	$(8.4 \pm 5.3) \times 10^{-7}$ mol/L	$(1.3 \pm 0.7) \times 10^{-6}$ mol/L

* $p < 0.05$ compared with unmarked vein.

1, B) in MB-marked versus unmarked vessels was reduced by $30\% \pm 10\%$ and $22\% \pm 8\%$, respectively. Compared with unmarked vessels, the maximal contraction of MB-marked saphenous vein, expressed in grams of tension, was reduced by $73\% \pm 8\%$, $53\% \pm 17\%$, $58\% \pm 13\%$, and $38\% \pm 12\%$ for phenylephrine (Fig. 2, A), serotonin (Fig. 2, B), angiotensin II (Fig. 2, C), and BHT (Fig. 2, D),

respectively. The same data, expressed as a percentage of maximal response to KCl, showed a reduction of $51\% \pm 21\%$, $45\% \pm 22\%$, $53\% \pm 14\%$, and $36\% \pm 20\%$ for phenylephrine, serotonin, angiotensin II, and BHT, respectively. Compared with unmarked vessels, those marked with MB showed a significant impairment in their ability to relax to all vasodilator agents used (Fig. 3). MB-marked veins demonstrated a sig-

Fig. 4. Maximal vasoconstrictor responses measured in grams of tension in saphenous veins comparing the effect on an experimentally exposed vein to 0.1% MB for 45 minutes abluminally only (*black column*, external; $n = 6/12$), abluminally and intraluminally (*stippled column*, external and internal; $n = 6/12$), or not exposed to MB (*open column*, control, $n = 6/12$). **A**, the constriction elicited by membrane depolarization induced by KCl, 100 mmol/L. **B**, Constriction elicited by exposure to PGF_{2α} 3 μmol/L. * $p < 0.05$ compared with control.

Table II. EC_{50} values for saphenous veins experimentally exposed to MB (0.1% for 45 minutes) as described in methods

Drug	EC_{50} MB free	EC_{50} EXT MB	EC_{50} INT/EXT MB
Phenylephrine	$(1.6 \pm 0.9) \times 10^{-6}$ mol/L	$(5.0 \pm 3.3) \times 10^{-7}$ mol/L	$(9.4 \pm 3.2) \times 10^{-7}$ mol/L
Serotonin	$(1.6 \pm 0.8) \times 10^{-7}$ mol/L	$(1.8 \pm 0.8) \times 10^{-7}$ mol/L	$(1.3 \pm 1.0) \times 10^{-7}$ mol/L
Sodium nitroprusside	$(3.3 \pm 1.07) \times 10^{-7}$ mol/L	$(6.4 \pm 3.8) \times 10^{-7}$ mol/L	$(1.5 \pm 1.5) \times 10^{-6}$ mol/L*
Acetylcholine	$(3.7 \pm 1.6) \times 10^{-8}$ mol/L	$(2.3 \pm 2.3) \times 10^{-8}$ mol/L	$(3.7 \pm 4.5) \times 10^{-8}$ mol/L

All data are from a single saphenous vein divided into three groups from a total of six separate patients. EXT, Vein exposed to MB externally only; INT/EXT, vein exposed to MB internally and externally.

* $p < 0.05$ compared with MB free vein.

nificant rightward shift in EC_{50} values for sodium nitroprusside and isoproterenol (see Table I). Evaluation of the nadir of the dilatory dose response curves demonstrated that relaxation was reduced by $54\% \pm 17\%$, $46\% \pm 17\%$, $53\% \pm 14\%$, and $78\% \pm 18\%$ for sodium nitroprusside (Fig. 3, A), acetylcholine (Fig. 3, B), isoproterenol (Fig. 3, C), and verapamil (Fig. 3, D), respectively, when data are expressed as a percentage of PGF_{2α} preconstriction. The same data expressed as grams of relaxation showed a similar reduction in the MB-marked vessels of $68\% \pm 12\%$, $68\% \pm 10\%$, $60\% \pm 7\%$, and $82\% \pm 6\%$ at the same point of sodium nitroprusside, acetylcholine, isoproterenol, and verapamil, respectively.

Veins experimentally exposed to MB. A total of 6 of 36 unmarked (from 3 men and 3 women) saphenous vein preparations were evaluated for vascular reactivity after experimental exposure to MB. Fig. 4 demonstrates MB-attenuated vasoconstrictor responses to both PGF_{2α} and KCl with the greatest impairment seen in the external/internal exposure

group. Compared with control vessels, vessels in the external MB group showed a $36\% \pm 16\%$ (Fig. 4, A) impairment of KCl constriction and a $33\% \pm 19\%$ (Fig. 4, B) impairment of PGF_{2α} constriction. Vessels in the internal/external MB group showed an even greater impairment of both KCl ($40\% \pm 16\%$, Fig. 4, A) and PGF_{2α} constriction ($59\% \pm 17\%$, Fig. 4, B). Phenylephrine and serotonin dose response curves comparing the three experimental groups indicate that internal/external MB exposure significantly attenuates the vasoconstrictor ability of saphenous veins (Fig. 5). Phenylephrine vasoconstriction in the external MB group was not significantly lower than in the control group. However, a trend toward a blunted response is evident (Fig. 5, A). For serotonin constriction, the external MB group was significantly impaired at 10^{-7} mol/L (Fig. 5, B). Compared with the control group, the maximal response of the external MB group to phenylephrine and serotonin was reduced by $28\% \pm 19\%$ and $48\% \pm 13\%$, respectively. For the internal/external

Fig. 5. Vasoconstrictor dose response curves for phenylephrine (A) and serotonin (B) obtained from saphenous vein ring preparations that were experimentally exposed to 0.1% MB for 45 minutes abuminally only (black triangles, external, $n = 6/12$), abuminally and intraluminally (black circles, external and internal, $n = 6/12$), or not exposed to MB (open circles, control; $n = 6/12$). Symbols on the X-axis depict EC_{50} values for the respective curve. * $p < 0.05$ compared with control.

MB group, the maximal response to phenylephrine and serotonin was reduced by $70\% \pm 6\%$ and $58\% \pm 16\%$, respectively.

Experimental exposure to MB also reduced the vasodilatory function of saphenous veins (Fig. 6). When the data are expressed as a percentage of $PGF_{2\alpha}$ preconstriction, MB did not significantly change acetylcholine relaxation although a clear trend is evident (Fig. 6, C). Expressed as a percentage of $PGF_{2\alpha}$ preconstriction, the internal/external MB group demonstrated significant attenuation of

sodium nitroprusside relaxation by $18\% \pm 11\%$ (Fig. 6, A) at the nadir of the dilator response curves. The EC_{50} value was also significantly shifted rightward, which indicates a decreased sensitivity of the internal/external veins to sodium nitroprusside (see Table II). When the data are expressed in grams of relaxation, both the internal/external and external MB groups are significantly inhibited from relaxing to sodium nitroprusside by $75\% \pm 6\%$ and $45\% \pm 12\%$, respectively (Fig. 6, B). Significant impairment ($67\% \pm 16\%$) of acetylcholine vasorelaxation in the internal/external group was also evident when the data were expressed in grams of tension (Fig. 6, D).

Discussion

The present study demonstrates that the use of MB as an extravascular surgical marker has the potential to impair vascular function, particularly when MB has access to both the adventitia and the endothelium. In practice, MB can access the endothelium when the isolated MB-marked vein is briefly placed in a storage solution until the time of transplantation. Leaching of MB on the adventitia into the storage solution allows for free diffusion of MB into the vessel lumen and hence the endothelium. The concentration of MB leached into the storage solution is not insignificant because the conventional 1% stock solution of MB is in excess of 500 times the dose required to irreversibly inhibit endothelium-derived relaxing factor.⁶ Further, results from this study indicate that in addition to the well documented inhibition of vasodilators,⁷ MB also diminishes the effects of other vasodilators and vasoconstrictors irrespective of the route of application. The current study was designed to evaluate numerous possible MB pathways through the use of a variety of vasoactive agents, but because MB caused an impairment of all agents tested, isolation of specific mechanisms is difficult. From a practical aspect, the mechanism of MB impairment of vascular function is perhaps less important than the clear documentation of an effect of MB at clinically relevant doses and exposure times.

The pharmacologic actions of MB have been extensively studied but remain poorly understood. MB is best known as a direct inhibitor of soluble guanylate cyclase, presumably via MB oxidation of the guanylate cyclase heme moiety.⁸ The ability of MB to inhibit those vasodilators that are thought to act by activation of guanylate cyclase is indirect evidence of inhibition of the enzyme by MB.⁷ This

Fig. 6. Vasodilator dose response curves for sodium nitroprusside (A and B) and acetylcholine (C and D) obtained from saphenous vein ring preparations that were experimentally exposed to 0.1% MB for 45 minutes abluminally only (black triangles, external, $n = 6/12$), abluminally and intraluminally (black circles, external and internal, $n = 6/12$), or not exposed to MB (open circles, control, $n = 6/12$). Because treatment affected precontraction, the data are expressed as both a percentage of PGF_{2α} precontraction (A and C) and as grams of relaxation (B and D). Symbols on the X-axis depict EC₅₀ values for the respective curve. * $p < 0.05$ compared with control.

includes all endothelium-dependent and -independent vasodilators that elicit vascular smooth muscle relaxation by either nitric oxide formation or nitric oxide release.⁷ Such a mechanism is consistent with the change in EC₅₀ values and inhibition of sodium nitroprusside and acetylcholine vasorelaxation observed in this study. However, an inhibition of guanylate cyclase by MB does not explain the impairment of isoproterenol or verapamil vasodilation. Nor does it explain the MB-mediated attenuation of vasoconstrictor responses seen in this study. The impairment of vascular reactivity in the current study seems to be global rather than specific for a given transduction system. MB-mediated reduction in the receptor-independent KCl vasoconstriction is evidence of the global nature of the impairment of vascular reactivity. This is not to say that inhibition of guanylate cyclase is an irrelevant mechanism contributing to the vascular impairment seen in this

study. Inhibition of this enzyme is probably occurring as evidenced by the shift in EC₅₀ values for sodium nitroprusside. However, other MB-mediated mechanisms of vascular impairment are likely dwarfing any guanylate cyclase effects.

MB is a more potent inhibitor of endothelium-dependent vasodilation mediated by acetylcholine than it is of endothelium-independent nitro dilators.^{9,10} This suggests that MB acts to inhibit vasodilation by mechanisms independent of guanylate cyclase. A recent study by Marczin, Ryan, and Catravas¹¹ suggests that MB inactivation of guanylate cyclase is only a minor pathway for the effects of MB. Rather, MB generates superoxide anion, which rapidly reacts with and inactivates nitric oxide as a vasodilator.¹² Marshall, Wei, and Kontos¹³ were the first to demonstrate that MB inhibits acetylcholine dilation via the production of a reactive oxygen species. Subsequently, Wolin and coworkers¹⁴ used

the cremaster microcirculation as a model to demonstrate that suffusion of superoxide dismutase readily inhibits the effects of MB on acetylcholine and nitric oxide vasodilation. MB is hypothesized to generate superoxide when the reduced form of MB autoxidizes¹³ or via an interaction with various dehydrogenases.¹⁵ Regardless of the exact mechanism, MB appears to produce significant amounts of superoxide in blood vessels. Moreover, a recent study demonstrated that MB generated superoxide in a dose- and time-dependent manner in the presence, but not absence, of cultured vascular smooth muscle cells.¹⁰

The generation of superoxide by MB is a potential mechanism of the vascular impairment seen in the present study. In vascular tissue, superoxide is converted to a variety of other potentially harmful reactive oxygen species including hydrogen peroxide, hydroxyl ion, and peroxynitrite.¹⁶ Countless studies have identified oxygen-derived free radicals as mediators of cellular damage,^{17, 18} including damage to vascular tissue.¹⁹ MB-induced oxidative damage has the potential to affect any number of pathways controlling smooth muscle or endothelial function²⁰ and would likely lead to a global impairment of vascular function as seen in this study. Whether this is the mechanism of MB impairment remains to be determined.

MB increases the spontaneous outflow and alters the storage of catecholamines from sympathetic nerves in vascular tissue. In vitro, MB causes a rise in vascular tone that is readily blocked with α -adrenergic agonists. Further, MB inhibits responses to both tyramine and electrical stimulation of adrenergic nerves of rabbit perfused ear arteries.²¹ Soares-Da-Silva and Caramona²² used canine mesenteric arteries to demonstrate that MB releases norepinephrine and dopamine, decreases accumulation of norepinephrine, and inhibits monoamine oxidase and catechol-*O*-methyl transferase. Collectively, these studies demonstrate that MB produces a prolonged sympathomimetic effect in vascular tissue. The MB-mediated reduction of adrenergic α_1 , α_2 , and β_2 responses in the present study could be explained by an adrenergic desensitization resulting from the prolonged sympathomimetic effect of MB. However, the magnitude of MB impairment was not greater in adrenergic than in nonadrenergic responses, suggesting other, more global mechanisms. Whether the effects of MB on the vascular adrenergic system are clinically relevant when MB is used as a marker dye is unknown, but in light of the results

of the current study, it seems that MB has any number of potential mechanisms by which to adversely affect blood vessels.

In conclusion, the current study has demonstrated that MB applied either luminally or abluminally can decrease both the vasoconstrictor and vasodilatory function of human saphenous veins by an unknown mechanism. These results, when coupled with other studies that have shown that MB produces superoxide, inhibits guanylate cyclase, increases platelet deposition,²³ and elicits a sympathomimetic response, suggest that the use of MB as a surgical marker is inappropriate until further studies can evaluate the potentially detrimental effects of MB.

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REFERENCES

1. Johnson PC, Sheppeck R. The dark side of methylene blue [Letter]. *Plast Reconstr Surg* 1989;83:1076.
2. Mazmanian GM, Bauder B, Pannier-Poulain C, et al. Pressor responses of rat isolated tail arteries to contractile stimulation after methylene blue treatment: effect of adventitial versus intimal entry. *J Vasc Res* 1993;30:250-6.
3. Bentz ML, Sheppeck RA, Macpherson T, et al. Vasospasm and platelet deposition in human arteries: effects of topical methylene blue. 1991;88:851-9.
4. Waud DR, Parker RB. Pharmacological estimation of drug-receptor dissociation constants: statistical evaluation. II. Competitive antagonists. *J Pharmacol Exp Ther* 1971;177:13-24.
5. Fleming WW, Westfall DP, De La Lande IS, Jellett LB. Log-normal distribution of equieffective doses of norepinephrine and acetylcholine in several tissues. *J Pharmacol Exp Ther* 1972;181:339-45.
6. Martin W, Villani GM, Jothianandan D, Furchgott RF. Selective blockade of endothelium-dependent and glyceryl trinitrate-induced relaxation by hemoglobin and by methylene blue in the rabbit aorta. *J Pharmacol Exp Ther* 1985;232:708-16.
7. Ignarro LJ, Kadowitz PJ. The pharmacological and physiological role of cyclic GMP in vascular smooth muscle relaxation. *Ann Rev Pharmacol Toxicol* 1985; 25:171-91.
8. Ignarro LJ, Burke TM, Wood KS, Wolin MS, Kadowitz PJ. Association between cyclic GMP accumulation and acetylcholine-elicited relaxation in bovine intrapulmonary artery. *J Pharmacol Exp Ther* 1984; 228:682-90.

9. Watanabe M, Rosenblum WI, Nelson GH. In vivo effect of methylene blue on endothelium-dependent and endothelium-independent dilations of brain microvessels in mice. *Circ Res* 1988;62:86-90.
10. Mayer B, Brunner F, Schmidt K. Inhibition of nitric oxide synthesis by methylene blue. *Biochem Pharmacol* 1993;45:367-74.
11. Marczin N, Ryan US, Catravas JD. Methylene blue inhibits nitrovasodilator- and endothelium-derived relaxing factor-induced cyclic GMP accumulation in cultured pulmonary arterial smooth muscle cells via generation of superoxide anion. *J Pharmacol Exp Ther* 1992;263:170-9.
12. Saran M, Michel C, Bors W. Reaction of NO with O_2^- : implications for the action of endothelium-derived relaxing factor. *Free Radic Res Commun* 1990;10:221-6.
13. Marshall JJ, Wei EP, Kontos HA. Independent blockade of cerebral vasodilation from acetylcholine and nitric oxide. *Am J Physiol* 1988;255:H847-54.
14. Wolin MS, Cherry PD, Rodenburg JM, Messina EJ, Kaley G. Methylene blue inhibits vasodilation of skeletal muscle arterioles to acetylcholine and nitric oxide via the extracellular generation of superoxide anion. *J Pharmacol Exp Ther* 1990;254:872-6.
15. McCord JM, Fridovich I. The utility of superoxide dismutase in studying free radical reactions. *J Biol Chem* 1970;245:1374-7.
16. Radi R, Cosgrove TP, Beckman JS, Freeman BA. Peroxynitrite-induced luminol chemiluminescence. *Biochem J* 1993;290:51-7.
17. Kukreja RC, Hess ML. The oxygen free radical system: from equations through membrane-protein interactions to cardiovascular injury and protection. *Cardiovasc Res* 1992;26:641-55.
18. Halliwell B. Free radicals, reactive oxygen species and human disease: a critical evaluation with special reference to atherosclerosis. *Br J Exp Pathol* 1989;70:737-57.
19. Rubanyi GM. Vascular effects of oxygen-derived free radicals. *Free Rad Biol Med* 1988;4:107-20.
20. Cohen RA. Pathways controlling healthy and diseased arterial smooth muscle. 1993;72:39C-47C.
21. Soares-da-Silva P, Gillespie JS. Indirect sympathomimetic effects of methylene blue in vascular tissues. *Blood Vessels* 1987;287-93.
22. Soares-Da-Silva P, Caramona MM. Effects of methylene blue on the uptake, release and metabolism of noradrenaline in mesenteric arterial vessels. *J Pharm Pharmacol* 1988;40:534-8.
23. Johnstone MT, Lam JY, Lacoste L, Baribeau J, Theroux P, Waters D. Methylene blue inhibits the antithrombotic effects of nitroglycerin. *J Am Coll Cardiol* 1993;21:255-9.